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OPTIMIZATION OF ENZYME TREATMENT FOR BANANA FIBER

AMRITA DOSHI[1] & ANJALI KAROLIA[2]

[1]BSR-SRF, Department of Clothing & Textiles, Faculty of Family & Community Sciences,
The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

[2]Guide & Head, Department of Clothing & Textiles, Faculty of Family & Community Sciences,
The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

ABSTRACT

Owing to environmental concern, it is imperative to utilize natural resources. Banana fibers, one of the minor cellulosic fibers are obtained from the pseudostem of banana plant. The pseudostems are normally discarded as biomass after harvesting the fruit. Banana fibers are potential textile fibers due to their excellent strength and lustre, however lacks spin ability due to stiffness. Lignin present in the bast fibers is one of the reasons that add to stiffness. Hence the present study is about softening banana fibers using enzymes. Four different enzymes were applied individually to optimize the concentration and conditions and a final treatment was standardized in combinations. The order of application of enzyme treatment as combination was also studied. Accordingly this paper also discusses the development towards the sequential and simultaneous mixed enzyme systems to accomplish softness.

KEYWORDS: Banana Fibers, Enzymes, Softening, Spin Ability

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Original Article

INTRODUCTION

Depleting natural resources, regulations on using synthetic materials, growing environmental awareness and economic considerations are the major driving forces to make the most of annually renewable resources such as biomass for various industrial applications.

Lignocellulosic agricultural by-products are a copious and cheap source for cellulose fibers. Agro-based biofibers have the composition, properties and structure that make them suitable for uses such as composite, textile, pulp and paper manufacture. By products produced from the cultivation of some food crops are the major sources of agro-based biofibers obtained from biomass.

Approximately 2×10^{11} tons of lignocellulosics are produced every year. Organic agricultural wastes (agricultural by products) are annually renewable, available in abundance and of limited value at present. These lignocellulosic by products can be a principal source for textile fibers and other industrial products. Primary lignocellulosic agricultural by products that are available in considerable quantity and at low cost are corn, wheat, rice, barley straw, sorghum stalks, coconut husks (coir), sugarcane bagasse, pineapple and banana. Using these crop residues for industrial applications could be an additional source of revenue for farmers, without affecting soil fertility.

Not all of these agricultural by products are commonly available across the world. Many are native to a particular region, depending on the climatic conditions required to grow the food crops. The value of the by product

depends on the availability and potential use of the by product. Banana is one of the most commonly grown fruit crop of the country. India produces about 26.217 Mmt of Banana from an area of 0.709 Mha. Major producing states are Tamil Nadu, Maharashtra, Karnataka, Gujarat, Andhra Pradesh, Assam and Madhya Pradesh.

Banana is one of the major fruits crops of Gujarat. Gujarat ranks sixth in the area cultivation of banana in the country with the average cultivation area of around 46,500 hectares with a production of 1.9 million tonnes and productivity of 42.7 tonnes/ha. This gives employment and income to millions of people engaged in its growing and trade. After the consumption of the fruit the plant is cut and thrown on the road side. This biomass is of great importance, as banana pseudostem is the basic raw material required for extraction of banana fibers and Gujarat has sufficient availability of the same to cater to the need.

The banana fibers extracted from the pseudostem of the banana plant are extremely strong, lustrous and long length filament fibers. Being lignocellulosic in nature, banana fibers are little stiff and less pliable. Therefore, the present study was undertaken with the objective to soften the banana fibers. Banana fiber being eco-friendly, environment friendly products i.e. enzymes were used to give softening treatment.

Chemical Composition of Fibers

The chemical composition of banana is inherent according to the particular needs of the plants. Cellulose, hemicellulose and lignin are the three main constituents of any lignocellulosic source, and the proportion of these components in a fiber depends on the age, source of the fiber and the extraction conditions used to obtain the fibers. Chemical composition of banana fiber is as follows: Cellulose 69.5, Hemicellulose 15, lignin 5.45, Pectin 0.5, Fats 1.5

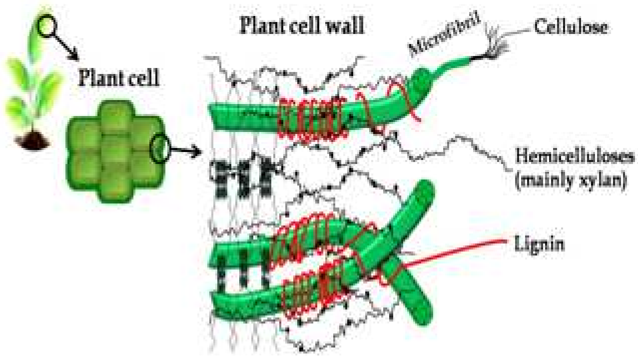

Figure 1: Positioning of Lignocellulosic Components in a Plant Cell Wall
(Source: http://www.intechopen.com)

Cellulose is the main structural component that provides strength and stability to the plant cell walls and the fiber. The amount of cellulose in a fiber influences the properties, economics of fiber production and the utility of the fiber for various applications.

Hemicellulose in plants is slightly cross linked. Hemicellulose usually acts as filler between cellulose and lignin as shown in Figure 1. Mechanically, hemicellulose contributes little to the stiffness and strength of fibers or individual cells. Hemicellulose is more easily hydrolyzed into sugars than cellulose.

Lignin is a highly cross linked molecular complex with amorphous structure and acts as glue between individual cells and between the fibrils forming the cell wall (Figure 1). Lignin provides plant tissue and individual fibers with

compressive strength and stiffens the cell wall of the fibers. The lignin content of the fibers influences the structure, properties, morphology, and flexibility of the fiber.

Enzymes

Minor fibers are receiving renewed attention in the quest to increase the use of sustainable fibers in all kinds of textile products, particularly for apparel. Several international organisations are promoting the innovative processes to soften the bast fibers using enzymes and thus removing the lignin. As banana is fast-growing, and can be cultivated using minimal to no pesticides or herbicides, and in addition, the enzymatic process is non-polluting and economical. Hence an eco-friendly product and process can be obtained.

MATERIALS AND METHODS

Material

For the present study banana fibers were obtained from Navsari Agricultural University. The plant variety was Grand Naine. The specifications for the fiber are shown in Table 1.

Table 1: Fiber Specifications

Property	Raw Banana Fiber
Length	80 – 110cms
Fineness	14.4 tex
Bundle Strength	40gms/tex

Methods

The fibers were treated with four enzymes – cellulase, hemicellulase, pectinase and lacase, for softening the fibers. The enzymes were procured from Rossari enzymes. The fibers were treated with four enzymes individually with varying time and concentrations. For all the treatments the M: L ratio was 1:40.

After optimisation of time and concentration of individual enzyme, combination treatments were conducted. The order of the application of the enzymes is also of immense importance. Two sets of combination treatment with different order of enzyme application were carried out (Refer Table 3).

Characterization

Weight Loss: The enzyme treatments were monitored by weight loss. Hence percent weight loss for every treatment was calculated.

Tensile Strength: To test the tensile strength of treated and untreated banana fiber Pressley Fiber – Strength tester was used. Bundle fiber strength was obtained by this method.

Hand Evaluation: To feel the softness of the fiber, the hand was analysed by subjective evaluation. A panel of 10 staff members of the department were asked to touch and feel the treated samples, and the samples were anonymously labelled.

RESULTS AND DISCUSSIONS

As mentioned earlier banana fibers are Lignocellulosic in nature and contains cellulose, hemicelluloses, lignin, pectins and therefore four enzymes were used in the study to target these major components of the fiber. The treatment involves the change of biomass, so that enzymatic hydrolysis of cellulose and hemicelluloses can take place.

Simultaneously the removal of lignin and disruption of the crystalline structure of cellulose is also observed.

Effect of Cellulase

Harmsen et al. (2010) posted that Cellulases are enzymes that hydrolyze β-(1-4) glycosidic bonds in cellulose. Cellulases can be broadly divided into three classes based on their catalytic action as endoglucanases, cellobiohydrolase, and β-glucosidase . Endoglucanases randomly attack the amorphous regions inside the cellulose chains on the surface of microfibrils and produce oligosachharides of varying lengths and create new chain ends for exoglucanases. Exoglucanases hydrolyse the cellulose chain from ends producing cellobioses or two units of glucose. Cellulose has both crystalline and amorphous regions and it is easier to hydrolyze amorphous regions in comparison to crystalline regions. Crystalline regions are resistant to attack by endoglucanases and the bonds cleaved are re-formed owing to stability of crystalline glucan chains. Therefore, synergism between endoglucanases and exoglucanases is vital for hydrolysis of cellulose. Exoglucanases chop off cellobiose units from newly created ends formed by endoglucanase, thereby preventing the reformation of bonds. The initial rate of enzymatic hydrolysis is relatively rapid and decreases over time. Therefore the time for treatment in the study was kept 60 minutes and 45 minutes as mentioned by the enzyme suppliers. Not much of difference was observed in terms of the feel of the fabric, thus time for all cellulase treatment was constant at 45 minutes. Temperature was maintained at 55^{o}C and pH at 4.5 – 5 by addition of acetic acid.

Four different concentrations of cellulase were studied, and it was observed that maximum weight loss was at 0.3% concentration, which supports the theory mentioned above for cellulose breakdown. The strength loss increased to great extend as the concentration was increased. Although strength loss was little more at 3.0% concentration of cellulase as compared to 0.2 %, but 0.3 % concentration (Figure 2) was optimized for the treatment, as it has better hand feel. Though 12.5% strength loss is high, but this loss can be acceptable because banana fibers are extremely strong fibers. Even after 12.5% strength loss the bundle strength of 0.3% cellulase treated fibers is 35gm/tex, which is still higher than cotton.

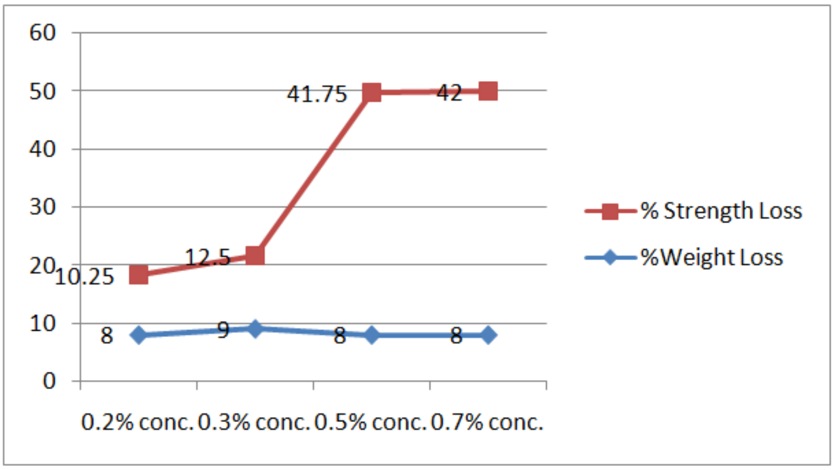

Figure 2: Effect of Cellulase on Banana Fiber

Effect of Hemicellulase

Xylan is the most abundant hemicellulose in the biosphere. Xylanases or hemicellulase were widely used in the pulp and paper industry since they increase lignin extractability. As mentioned above in Picture 1, hemicellulase acts as

fillers between cellulose and lignin, therefore breakdown or removal of hemicelluloses will aid in softening the fibers.

For the present study 4 different concentrations of hemicellulase were taken. Time for all the treatments was 60 minutes at a temperature of 40°C, maintaining the pH at 4.5 – 5 by addition of acetic acid. The percent weight loss decreased after 1% concentration which could be understood as the enzyme activity of hemicelluloses removal becomes stagnant after 1% concentration treatment. The strength loss is higher as compared to cellulase treated fibers. This could be attributed to the fact that hemicellulose starts degrading earlier than cellulose. Owing to this strength loss of 23% at 1% concentration, experiments were conducted with lower concentration of hemicellulase. Barely any smooth feel was observed at lower concentration treatments and therefore pertaining to weight loss, strength loss and feel of the fiber 1% concentration was optimized for the study (Figure 3).

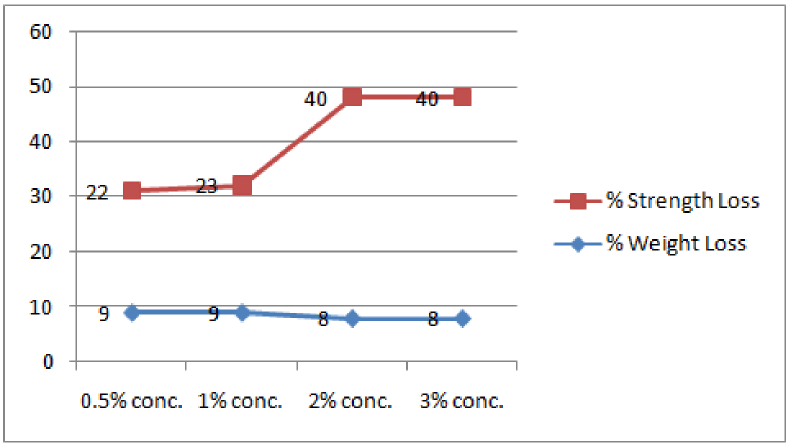

Figure 3: Effect of Hemicellulase on Banana fiber

Effect of Lacase Enzyme

Lignin is the most complex natural polymer. It is an amorphous three-dimensional polymer with phenylpropane units as the predominant building blocks. As has been mentioned by Harmsen et al. (2010) Laccase moreover degrades phenolic lignin and phenolic lignin model compounds and nonphenolic substrates are not oxidized directly. The phenolic lignin structures account for 10–20% of the lignin substructures in the plant cell wall, laccase can therefore not play a role as a delignifying agent completely.

Lignin is a highly crosslinked molecular complex acting as glue between cellulose and hemicelluloses. Predominantly due to lacase, bast fibers obtain such high strength. For the present study banana fibers were treated with four different concentrations, keeping 30 minutes time of treatment at 55^0 C and pH 5 – 6. Weight loss was less as compared to other enzyme treatment, this could be stated due to the fact that lacase alone acts on only phenolic lignin content. Strength loss is also less in the lower concentrations of lacase, with the increase in concentration sever strength loss is observed. However as the concentration increased the hand of the fibers was getting softer but due to strength loss, higher concentrations could not be optimized. (Figure 4)

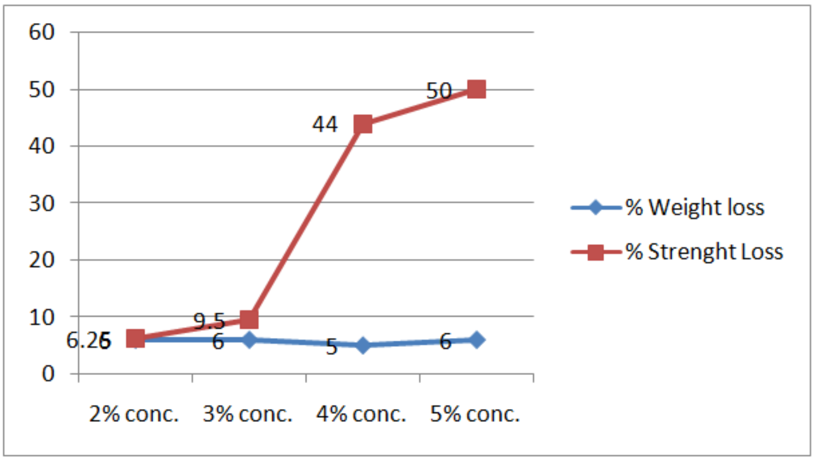

Figure 4: Effect of Lacase on Banana Fiber

EFFECT OF PECTINASE ENZYME

Pectinase is a mixed enzyme that effectively removes pectins, fats and waxes. Pectins, fats, waxes account for 2 to 3 percent of the total composition, but their removal is essential for the soft feel of the fibers. Three different concentration were tried for the study, keeping time 15 minutes, temperature 55°C and pH 5.5 as constant. Weight loss is similar as other enzyme treatment, but percent strength loss is high even at lower concentration, like hemicellulase (Table 5). Results of hand evaluation suggested 0.7% concentration as the softest feel amongst all three, and were thus optimised.

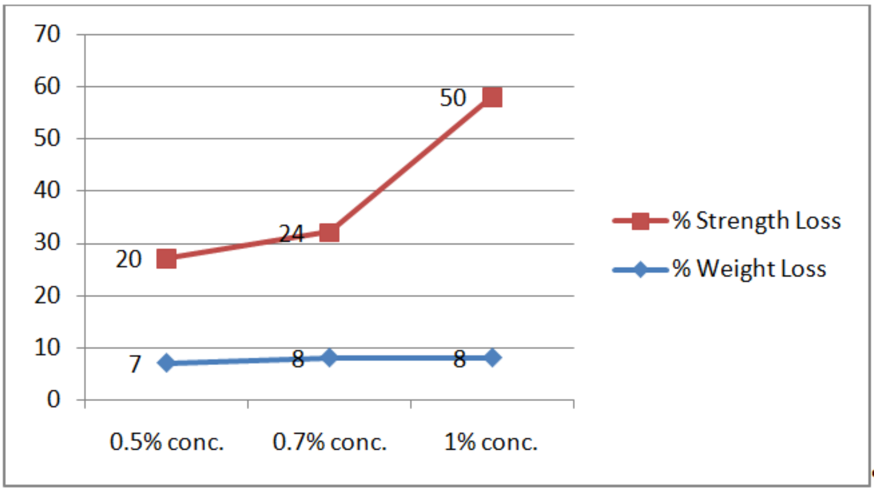

Figure 5: Effect of Pectinase on Banana Fiber

Considering the strength loss and weight loss and the subjective evaluation by hand, following parameters were optimized for enzyme treatment mentioned in Table 2

Table 2: Optimised Conditions for Enzymes Treatment of Banana Fiber

Enzyme	Concentration	Time	Temperature	pH
Lacase	3%owf	30 min	55°C	5-6
Hemicellulase	1%owf	60 min	40°C	5.5-6
Cellulase	0.3%owf	45 min	55°C	4.5-5
Pectinase	0.7%owf	15 min	55°C	5.5

After optimization of the parameters of individual enzyme, combination of enzymes was experimented. Two sets were made; set I comprised of enzymes combined on the basis of time of treatment and pH. The set II was made considering the structure of the plant cell Table 3.

Table 3: Combination Treatment

Set I	Set II
Lacase and cellulase treatment for 40 min	Lacase treatment for 45 min
Pectinase treatment for 15 min	Hemicellulase treatment for 60 min
Hemicellulase for 60 min	Cellulase treatment for 30 min
	Addition of Pectinase in Cellulase treatment bath after 15 min

Set II proved to be a promising strategy. The percent weight loss was more as compared to set I, which indicated that more impurities were removed by second set of treatment. Elisa M. Woolridge (2014) mentioned that lignocellulose is a class of biomass consisting of three key compounds cellulose, hemicellulose and lignin. It also includes water and a small amount of proteins and other compounds, which do not participate significantly in forming the structure of the material. Inside the lignocellulose complex, cellulose retains the crystalline fibrous structure and it appears to be the core of the complex. Hemicellulose is positioned both between the micro- and the macrofibrils of cellulose. Lignin provides a structural role of the matrix in which cellulose and hemicellulose is embedded.

When activity of lacase and hemicellulase begins, they start to loosen the complex structure. When lacase removes lignin, consequently links between the Lignocellulosic complex also begins to break. Also after lignin removal from the fiber surface, reagent penetration in subsequent stages is made easy and the further enzyme activity is strengthened. Once the complex structure is targeted, it is easy to remove lignin, hemicelluloses, pectins and cellulose breakdown in the crystalline region. This is also confirmed by analysis of chemical composition of the two treated fibers, which will be shown in the part II of the paper.

Table 4: Effect of Combined Enzyme Treatments

Treatment condition	Weight Loss (%)	Strength Loss (%)
Set I	7.5	20
Set II	8.5	17.5

Besides this, the percent strength loss was less in set II, which is significant result as required (Table 4). Although the percent loss of set II is 17% which is high in itself but can be acceptable as for softening because even after 17% strength loss the bundle fiber tensile strength is 33gms/tex, which is acceptable. To use the enzyme treated banana fiber as blend with other fibers, 33gms/tex can be rated as good to very good category of tensile strength. Another fact associated with strength loss during enzyme treatment on banana fiber is that the fibers will lose strength because the treatment conditions are acidic. Most cellulosic fibers loose strength in acidic conditions.

The result of hand evaluation test was 100% in support of set II treatment, i.e. banana fibers treated with set II enzyme were softer than set I. Besides this set II fibers tex was improve from 14.4 tex to 11.5 tex.

CONCLUSIONS

Banana fibers can be softened by using enzymes. The percent weight loss of the fibers supports that unwanted material is removed by the enzymes. The combination of enzymes in a specific order (first lacase followed by hemicellulase and then cellulase and pictinase) gave better results in terms of weight loss and the feel of the fiber. In

Lignocellulosic fibers, lignin acts as glue between cellulose and hemicelluloses. Here banana fibers are first treated with lacase which removes lignin and simultaneously sets hemicellulase free. When the other enzymes are applied after lacase, the unwanted material can be removed with ease as the complex lignocellulosic structure has already loosened up. Thus, the principle of first targeting lacase removal followed by other unwanted impurities gave better results.

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